Crepidoodinium australe n. sp., an ectocommensal dinoflagellate from the gills of Sillago ciliata, an estuarine fish from the New South Wales coast of Australia

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ABSTRACT: Crepidoodinium australe n. sp., an ectoparasitic dinoflagellate, is described from the gills of Sillago ciliata (sand whiting) from the coast of New South Wales, Australia. Large, flat trophonts with a pointed apex, up to 820 x 235 µm in size, are attached to the gill filaments. Grown trophonts detach from the host, sink to the bottom, round up, and secrete a cyst envelope. Inside, the trophont divides into dinospores of Gyrodinium type, 17 x 12 µm, which migrate to new hosts. C. australe differs in morphology, hosts and area of distribution from C. cyprinodontum, the only species known of the genus. C. australe is ectocommensal. It has a strongly developed plastid system and is attached to the surface of the epithelial cells of the gills by means of tiny cytoplasmic projections, rhizoids. Thus a firm adherence to the host is ensured. However, no obvious injury is inflicted upon the host cells. C. australe is characterized by numerous pits in the surface theca, on the bottom of which are clusters of cisternae, each cluster being comparable to a small pusular system. The large nucleus of the trophont lacks condensed interphase chromosomes and reveals an internal network of canalicules representing numerous invaginations of the nuclear envelope.

INTRODUCTION

Species of several genera of dinoflagellates live in association with fish hosts. In Amyloodinium spp., a fine structure study (Lom & Lawler 1973) confirmed their pathogenic nature by showing the destruction of the host epithelium by the parasites. Amyloodinium sp. has been known since the papers of Brown (1934) and Nigrelli (1936, 1940) to endanger fish kept in marine aquaria and to be a pest in marine aquaculture (Lawler 1977, 1979, Ghittino et al. 1980, Paperna 1980, 1984, Baticados & Quinitio 1984, Barbaro & Francescon 1985). In freshwater, Piscinoodinium pillulare (Jacobs 1946, Schaperclaus 1951, Shaharon-Harrison et al. 1990) similarly damages epithelial cells (Lom & Schubert 1983). Heavy pathogenic action has also been shown for dinoflagellate organisms of uncertain taxonomy causing cutaneous lesions in Canadian sticklebacks Gasterosteus aculeatus (Reimchen & Buckland-Nicks 1989, Buckland-Nicks et al. 1990) and in Tribolodon hakonensis in Japan (K. Nagasawa unpubl.). The species Ichthyodinium chabelardi Holland & Cachon, 1953 infecting the oocytes of sardines was found just once (Holland & Cachon 1953). The genus Oodinioides Reichenbach-Klinke, 1970, described as occurring on the body surface but also in the internal tissues, probably does not exist (Lom 1981).

In contrast to the organisms named above, the thus far monotypic genus Crepidoodinium Lom & Lawler, 1981 in Lom (1981) seems to be an ectocommensal rather than a true ectoparasite, as the trophonts are fully photosynthetic and no serious damage is inflicted upon the epithelial cells of the host to which the trophonts are attached (Lom & Lawler 1973). While examining protozoan parasites from the New South Wales coast in Australia, we came across Crepidoodinium trophonts on the gills of an estuarine fish, Sillago ciliata. The evidence obtained is in favour of naming it a new species, which we describe as Crepidoodinium australe n. sp.
MATERIAL AND METHODS

In the course of a survey of protozoan parasites of Australian fishes, 24 specimens of sand whiting *Sillago ciliata* (fam. Sillaginidae) were examined in September and October 1990. They were collected at 2 localities, in the estuary of the Arrawarra Creek north of Coffs Harbor (New South Wales) and at Nambucca Heads south of Coffs Harbor. Live fishes were brought to the laboratory in the original water and, if not examined at once, kept in aquaria with sea water.

Live trophonts were observed. For transformation of trophonts into dinospores, sea water supplemented with streptomycin (1000 μg ml⁻¹) was used. Gill filaments with attached trophonts were fixed for 60 min in cold 2 % osmic acid in 0.1 M cacodylate buffer and embedded in Epon-araldite. Ultrathin sections were double stained with uranylacetate and lead citrate and examined under a Philips 420 B electron microscope at 80 kV accelerating voltage.

RESULTS

Light microscopy

In fishes from the Arrawarra Creek estuary, 5 out of 6 were infected with an intensity of up to ca 50 trophonts per gill arch, while out of 18 fishes collected at Nambucca Head, 9 were lightly infected, with 1 to 2 trophonts per gill arch.

The trophonts (Figs. 1 & 4 to 6) were found exclusively on the gill filaments, not on the skin. They were conspicuous at first inspection because of their large size and vividly green colour due to numerous chloroplasts. They were flat, leaf-like in side view and broad, apically pointed and basally extending into a flat holdfast surface in the frontal view. Their length was up to 820 μm, with width in frontal aspect up to 235 μm. The surface bore one to many deep furrows reflecting longitudinal surface folding. At higher magnification, tiny circular structures could be seen distributed over the entire theca (Fig. 5).

The holdfast surface, spread to a width of up to 530 μm and closely attached to the filament or secondary lamella epithelium, is cleaved into large and small projections, lobed and finger-like, bearing tiny extensions with hyaline cytoplasm without chloroplasts. A disc-shaped nucleus, up to 140 μm in diameter, is located at mid-length of the body.

Grown trophonts cling very firmly to the gill filaments. If they are gently removed from the gills by a surgical knife, or if the filament bearing them is clipped off and the trophonts are put in sea water, they engage in dinospore formation. The trophonts still on the fila-

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**Fig. 1** *Crepidodinium australis*. A: Trophont seen from the broad side; B, C: difference in trophont shape in side and frontal view respectively; D, E: dinospores. N: Nucleus
ments detach themselves spontaneously within several hours. They become round, produce a hyaline, thin cyst wall, become what we may call a tomont and start a series of binary fissions. At a temperature of about 23°C, after 24 h, the reproductive cyst contains a large number of dividing cells or tomites (Figs. 1D, E, 2 & 3). Within 48 h, a large number of flagellated dinospores are formed which then escape from the cyst wall. The dinospores swim quickly in the water for about 3 h after which time they die off. The short survival time is explained by not quite suitable conditions in the laboratory experiment.

The average size of live dinospores is 17 × 12 μm. They are ellipsoidal in shape, broader anteriorly. The sulcus groove begins in the posterior third or fourth of the body length at which point the 2 flagella are inserted (Fig. 1D, E). The cingulum groove branches off the sulcus at some distance from its origin and then spirals up around the cell forming a turn of about 360°. Its upper end is thus located approximately above the sulcus; however, it is not interconnected with it by any longitudinal furrow. A spherical nucleus, averaging 5 μm in diameter, is located in the posterior half, i.e. in the hypocone. When observed alive, no chromosomes can be discerned. Anteriorly, i.e. in the epicone, there are 1 to 3 ellipsoid chloroplasts. A mass of starch grains is closely packed in the posterior body half, where they form a mosaic-like pattern beneath the theca. Only a few are in the epicone cytoplasm.

The transformation of the dinospore into the trophont could not be examined.

**Electron microscopy**

**Theca.** It consists essentially of 3 unit membranes (Figs. 7 & 8). The outermost one, the cell membrane, covers the entire surface of the body; the other 2 membranes limit the very flat thecal alveoli, closely abutting upon each other. The alveoli closely subtend the outer cell membrane, across the gap of only 100 nm. There is usually a fourth membrane closely apposed on the inner surface of the proximal (bottom) alveolar membrane (Fig. 7). The alveoli are closely packed in the posterior body half, where they form a mosaic-like pattern beneath the theca. Only a few are in the epicone cytoplasm.

Associated with the theca are anterior tips of the trichocysts (Fig. 8) which approach the theca from beneath and thus seem to be wedged just beneath the outer cell membrane. Trichocysts are formed within the cytoplasm. They are single membrane bounded organelles, cylindrical to slightly spindle-shaped, with a markedly attenuated anterior end (see Fig. 12), complying essentially with other dinoflagellate trichocysts (also termed akontobolocysts; see Dodge 1987).

The circular structures seen with the light microscope in the theca appear under the electron microscope to be rather deep cylindrical (Fig. 9) or conical depressions of the theca. At the bottom of these pits is another, central depression lined only by the outer cell membrane (Fig. 10). In this membrane open elongated electron lucent saccules about 0.7 to 1.6 μm in length. The wall of some of the saccules may consist of 2 unit membranes. The membrane of the central depression is also pierced by pores with opaque side walls (Fig. 11). Among the lucent saccules are found vesicles filled with a moderately dense, finely granular substance (Fig. 11). The complex of these saccules and vesicles may be partly encircled by sheets of microtubules (Fig. 10) which may also form aggregations deeper in the cytoplasm.

**Cytoplasm.** It has a lacunar appearance, with vacuolar spaces separated by cytoplasmic trabeculi. In these cytoplasmic regions, Gogi apparatuses, inconspicuous mitochondria with poorly developed tubular cristae (Fig. 13) and fibrillar strands can be seen. These fibrillar bundles (Fig. 18), extending in various directions and sometimes wavy, are especially numerous in the vicinity of the nucleus. To some extent they are reminiscent of microtubules; their thickness is ca 40 to 50 nm. Throughout the cytoplasm are distributed rod-like bacteria localized directly in the cytoplasm and hence symbiotic (Figs. 14 & 16). In some regions they are present in larger aggregations suggesting intensive division.

The chloroplasts (Figs. 13 & 14) are typically oval, their envelope consisting of 3 membranes, with lamellae made up of 3 thylakoids.

There are 2 kinds of pyrenoids, simple internal ones, which lie between the chloroplast lamellae, and external, stalked ones, bound by the chloroplast envelope and attached to several chloroplasts (Fig. 14).

Some chloroplasts of irregular shape have only a few peripherally located lamellae while their interior is occupied by vesicles of various size, usually of lucent contents (Fig. 15).

**Nucleus.** In the interphasic trophont nucleus, the double nuclear envelope, equipped with pores shows deep invaginations all over its surface (Figs. 17 & 18). These invaginations become separated from the surface and can be seen as irregular elongated vesicles extending inside the nucleus in chain-like configurations. One can assume that this network is 3-dimensional as it delimiting small compartments in the nucleus. There are no permanent dinoflagellate chromosomes. At one end (Fig. 17) the nucleus is almost uniformly filled with what appears as a finely granular (fibrillar?) mass, completely absent in other regions of the nucleus, from which it seems to be separated by a wall of vesicles. The rest of the nuclear contents is filled with fibrillo-granular structures (Fig. 19) ranging from
small dense aggregations to larger, extremely dense bodies. In addition, there are moderately dense structures, tentatively interpreted as nucleoli (double arrows, Fig. 19). In the absence of cytochemical reactions, which could not be performed for technical reasons, and since we did not observe the division process, we could not interpret these structures properly.

**Holdfast surface and attachment to host.** The sole of the holdfast surface is branched into numerous major or minor ramifications. They are covered with the theca which is often quite wrinkled. At the tip of these projections there is an area devoid of theca, with only the outer cell membrane covering the cytoplasm. This is the point where the cytoplasm bulges out to produce cone- or finger-like tips (Figs. 20, 21), the actual rhizoids, which contact the outer cell membrane of the gill epithelial cells. Numerous cytoplasmic trabeculae converge from inside of the cell into the cytoplasmic bulge; immediately above it, there are no cytoplasmic organelles. If the gap in the theca is wide, the large cytoplasmic evagination may form 2 (Fig. 21) or more finger-like rhizoids; if it is narrow a single cone-like rhizoid protrudes from it. The rhizoids are not permanent formations. In a trophont detaching its holdfast surface from the gill epithelium, they can be fully retracted into the cell body (Fig. 22).

At the point of contact of the rhizoid with the epithelial cell, there is a corresponding indentation in the surface of the latter. Membranes of both the trophont and the epithelial cells stay intact, forming at their interface a ring of gap junction. Beneath the epithelial cell membrane, bundles of microfibrils converge. The regions of the host cell periphery with attached rhizoids are markedly elevated, obviously due to the pull of the rhizoids. No pathological changes could, however, be noticed.

**DISCUSSION**

**Species identity**

Thus far, the genus *Crepidooidinium* has been monotypic, with a sole species *Crepidooidinium cyprinodontum* (Lawler 1967) living on the gills of *Cyprinodon variegiatus, Fundulus heteroclitus, F. luciae, F. majalis and Lucania parva* (all Cyprinodontidae) at the Virginia, USA, coast of the North Atlantic (Lawler 1967, 1968a, b). The trophont of *C. cyprinodontum* is slightly smaller, up to 673 ± 152 µm, its distal free end gradually tapers, and is said to be slightly flattened. In the present species, the trophont is markedly pointed at its end and is considerably flat. The dinospores of *C. cyprinodontum*, not illustrated in the original description of Lawler (1967), were described as being smaller, 5–9 × 2–3 µm. While the trophont size in parasitic dinoflagellates depends on the state of nutrition and on environmental factors, the size of dinospores tends to be subject to much smaller variation, like in other protozoa with a similar cell cycle (e.g. *Ichthyophthirius* or *Cryptocaryon*). The morphology of *C. cyprinodontum* dinospores cannot be interpreted safely from the rather hazy description.

While *Crepidooidinium cyprinodontum* lives in 5 host species of the family Cyprinodontidae (Lawler 1967, 1968a, b), *Crepidooidinium australe* is obviously strictly host specific. In the course of a very large survey of Australian coastal (1862 fish of 46 species) and deepwater fish (1563 fish of 67 species), Rohde (1988) found *C. australe* only on whiting.

Considering all the features of the present species combined, we cannot identity it as *Crepidooidinium cyprinodontum*: trophonts are of a different shape; dinospores differ in size quite markedly; hosts belong to different families, although the habitat is similar; and the area of distribution is quite different. Therefore we propose to establish a new species for it, *Crepidooidinium australe* n. sp., the name being derived from its area of occurrence.

This distinction is supported by some features found at the ultrastructural level. The presence of endosymbiotic bacteria in the present species may be a feature of a certain population only. However, the thin thecal alveoli in *Crepidooidinium cyprinodontum* contain delicate membranes, while in *Crepidooidinium australe* they are filled with amorphous substance. The trichocysts in *C. cyprinodontum* have a distinct fibrous anterior part, while such fibers are in most cases difficult to distinguish in *C. australe*.
In the original description (Lawler 1967) the type of the dinospore was not specified. Our study has shown that they are of the *Gyrodinium* type and thus markedly different from the *Gymnodinium* type dinospores of *Amyloodinum* and *Piscinooodinium*. All 3 genera were assigned to 1 family, Oodinidae, according to Cachon & Cachon (1987), or Protoooidiaceae, according to Taylor (1987). However, the different types of dinospores do not necessarily contradict this classification since, curiously enough, the type of dinospore—gymnodiniform, gyrodiniform or cochlodiniform—thus far is deemed to be of great importance in supergeneric classification of these parasites (Cachon & Cachon 1987). The dinospore morphology is not conservative enough to be a taxonomic criterion in the parasitic dinoflagellates.

**Ultrastructural features**

The absence of condensed interphase chromosomes in the nucleus of *Crepidoodinium australe* is at variance with its presence in trophonts of some other parasitic dinoflagellate species such as *Haplozoomon axiothelae* (Siebert & West 1974) or *Piscinooodinium pillulare* (Lom & Schubert 1983). It confirms, however, the fact that such interphase chromosomes are missing in dinoflagellates, which include in their life cycle a relatively long period of growth, during which the nuclei increase in size without division (Raikov 1982), such as in the free living genus *Noctiluca* or in trophonts of uninucleate parasitic forms. It has been suggested, however, that in the dinospores of the parasitic forms the chromosomes, undetectable in trophonts, become condensed again in the dinospores, as e.g. *Dubosquella aspida* (Raikov 1982). The same type of nucleus is found in *C. cyprinodontum*; thus the genus complies with the general rule that vesicular nuclei are typical of parasitic dinoflagellates with hypertrophic growth during the trophont phase.

Another feature of the nuclear structure characteristic of *Crepidoodinium* and other parasitic dinoflagellates are the deep invaginations of the nuclear envelope. To be seen to some degree already in free living species (*Conyaulax polyedra*; Schmitter 1971), in *Blastodinium* they reach considerable development. They constitute a system of branched canalicules called plasmodendrites (Soyer 1971) pervading the nuclear structure inside. In *Crepidoodinium*, these plasmodendrites are richly developed and in addition to pervading the whole nucleus they constitute a net-like wall separating a peripheral nuclear segment with a special structure. The understanding of the role of these nuclear components can be perhaps elucidated by observation of their behaviour during mitosis.

At variance with *Amyloodinum* and *Piscinooodinium* (Lom & Lawler 1973 and Lom & Schubert 1983 respectively) we failed to observe in the attached trophont any remnants of the sulcal groove, let alone the flagellum. Also, no trace of a pusular system similar to that found in these 2 genera could be found in *Crepidoodinium*. However, the pits in the theca, evenly distributed on the surface of the trophont with a system of vesicles at their bottom resembling the pusular vesicles in e.g. *Amphidinium hermanii* (Dodge 1972) or *A. carteri* (Dodge & Crawford 1968), can be considered as homologous to pusules. They seem to constitute an extensive multiple pusular system compensating its small size—as compared e.g. with the elaborate pusular system in *Oodinium* (Cachon et al. 1970)—by the great number of these organelles.

**Relation to the host**

*Crepidoodinium cyprinodontum* was classified as a symbiotic ectocommensal in view of 2 main findings: the strongly developed plastid system and the missing indication of phagocytosis of the host cell components suggest photosynthesis as the main mode of nutrition; the absence of marked damage to the colonized host cells shows that the trophonts cause little—if any—damage to the host gills except perhaps for impairing the water flow in heavy infections. The same applies to *Crepidoodinium australe*. At the point of contact with the rhizoid tip, the cell membrane of the host epithelial cell is slightly modified, subtended by a thin layer of more opaque material from which microfibers and/or microtubules radiate into the host cell cytoplasm, but it never loses its integrity. This cell junction, which can be compared to a gap junction, is very firm as evidenced by the attached peripheral part of the host cell which is pulled out into an elevated

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Figs. 10 to 15. *Crepidoodinium australe*. Fig. 10. Bottom of the thecal pit with pusule-like saccules (*•*), with vesicles containing lucent substance (small arrows) and a microtubular sheet (large arrow). Arrowheads: points at which the surface theca stops. Fig. 11. Bottom of another thecal pit showing a pore with opaque wall in the simple surface membrane (arrow). Fig. 12. A trichocyst with indistinct fibrous structures in the apex. Fig. 13. Part of endoplasm with mitochondria (arrow) and a chloroplast with 3 membrane envelope. Fig. 14. A double stalked pyrenoid next to endosymbiotic bacteria (arrows). Fig. 15. An irregular chloroplast filled with a variety of vesicles. S: starch grains. In Figs. 10 to 14 scale bar = 0.5 μm, in Fig. 15 scale bar = 2 μm.
cone (Fig. 22). This is at variance with attachment of C. cyprinodontum where such a phenomenon was not detected.

In small cyprinodontid fish, hosts of Crepidoodinium cyprinodontum, the gill opercula are thin enough to transmit sufficient light for photosynthesis. In larger...
Sillago ciliata the gills are quite massive and yet the amount of sunlight passing through is sufficient for efficient photosynthesis as evidenced by the presence of starch grains in the ectocommensal. A different situation apparently exists in the chloroplast-bearing trophonts of Piscinoodinium found in internal body tissues of fish (Reichenbach-Klinke 1956, Schubert 1959).

Unlike Amyloodinium and Piscinoodinium, Crepidoodinium is restricted to the gills. Reasons for this may be the better mechanical protection of the large trophonts in the branchial cavity as compared to the exposed position on the skin, and perhaps dependence on the host metabolites excreted through the gills. One might speculate that an indication of such a possible substance uptake is the enormous number of pusule-like organelles on the trophont surface, unique to this exclusively gill-inhabiting genus.
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